Depression, anxiety and cellular aging: does feeling blue make you grey?

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Chapter 1

General introduction

Besides definitions provided by science or symptom clusters in diagnostic manuals, the real essence of depression and anxiety may be best captured by someone who has actually faced it. Andrew Solomon, writer and Professor of Clinical Psychology at Columbia University Medical Center, has gone through several episodes of depression and anxiety. He is the author of numerous books (e.g., The Noonday Demon: An Atlas of Depression (1)) and essays in which he examines depression in cultural and scientific terms, but also according to his personal struggles. The quote below is adapted from his recital "Depression, the Secret We Share" (2) and provides a powerful description of the burden that depression and anxiety can be.

"I found myself losing interest in almost everything. I didn't want to do any of the things I had previously wanted to do, and I didn't know why. The opposite of depression is not happiness, but vitality, and it was vitality that seemed to seep away from me in that moment. Everything there was to do seemed like too much work. I would come home and I would see the red light flashing on my answering machine and instead of being thrilled to hear from my friends, I would think, "What a lot of people that is to have to call back." Or I would decide I should have lunch. And then I would think that I would have to get the food out and put it on a plate and cut it up and chew it and swallow it, and it felt to me like the Stations of the Cross. One of the things that often gets lost in discussions of depression, is that you know it's ridiculous. You know it's ridiculous while you're experiencing it. You know that most people manage to listen to their messages and eat lunch and organize themselves to take a shower and go out of the front door and that it's not a big deal. And yet, you are nonetheless in its grip and you are unable to figure out anyway around it. And so, I began to feel myself doing less, thinking less and feeling less. It was a kind of numbing.

And then the anxiety set in. If you told me that I have would to be depressed for the next month, I would say as long as I know it will be over in a month I can do it. But if you said to me, you have to have acute anxiety for the next month, I would rather slit my wrists than go through it. It was the feeling all the time like that feeling you have if you are walking and you slip or trip and the ground is rushing up at you, but instead of lasting half a second the way that does, it lasted for six months. It is a sensation of being afraid all the time, but not even knowing what it is that you're afraid of. And it was at that point that I began to think that it was just too painful to be alive and that the only reason not to kill oneself was so as not to hurt other people."

1. DEPRESSIVE AND ANXIETY DISORDERS

1.1 Clinical picture

After reading Andrew Solomon's narrative, one cannot but conclude that being depressed or anxious can cause immense suffering, and therefore has a detrimental impact on quality of life and daily functioning. The World Health Organization (WHO) indeed states that depressive and anxiety disorders are leading causes of disability, and depression is amongst the largest contributors to the global disease burden (3). Lifetime prevalence rates are around 15%, hence one out of six adults will experience a depressive or anxiety disorder during their lives (4,5). The depressive and anxiety disorders studied in this thesis are among the most prevalent and include major depressive disorder (MDD), dysthymia, social phobia, panic disorder, agoraphobia and generalized anxiety disorder. These disorders often co-occur in the same person, which is illustrated by high comorbidity rates of over 60% (6). Each disorder is classified by the American Psychiatric Association according to a cluster of symptoms which are published in the Diagnostic and Statistical Manual of Mental Disorders (DSM; fourth edition in this thesis). The DSM-IV offers a common language for clinicians, researchers and policy makers worldwide (7).

MDD is defined as the presence of a depressed mood and/or a loss of interest during the largest part of the day for at least two consecutive weeks. This is often accompanied by several, if not all, other symptoms which include fatigue, increased or decreased appetite, sleep problems, cognitive symptoms such as difficulty with concentration or making decisions, feelings of guilt and worthlessness and thoughts of death or suicide. Dysthymia is a milder yet more enduring type of depression that is used to describe people who have a continuous (mildly) depressed mood for at least two years. Generalized anxiety disorder is, in turn, a condition in which persons constantly and disproportionally worry about a variety of topics or situations. This excessive worrying leads to symptoms such as fatigue, agitation, restlessness and the inability to control the anxiety, causing a constant anticipation of problems and difficulties. In persons with social phobia the worrying is mainly focused on social situations, during which persons experience an overwhelming anxiety and excessive self-consciousness. This social anxiety may lead to blushing, stammering and eventually avoidance of social situations and substantial social or occupational impairment. Panic disorder is characterized by recurring unprovoked panic attacks, during which persons experience extreme anxiety. Panic attacks typically last about ten minutes and often include rapid heartbeat, dizziness, nausea, excessive sweating and a sense of altered reality. Experiencing panic attacks may lead to behavioral changes due to the apprehension of having other attacks, such as avoidance of situations that might provoke an attack. This is why panic disorder is often accompanied by agoraphobia where persons experience

anxiety in environments they perceive as uncomfortable or unsafe such as wide-open spaces, unfamiliar places, shopping malls or public transport.

It should be noted that classifying a person's symptoms according to the DSM-IV may not always match real-life situations. As the majority of persons with depression also has an anxiety disorder, and vice versa, combinations of depressive and anxiety symptoms differ considerably between persons. Further, depressive and anxiety disorders might not be totally distinct conditions but rather overlapping concepts sharing factors that surpass traditional diagnoses. The considerable overlap may be due to similar etiologies. Depressive and anxiety disorders share the same risk factors which include heritability, adversities in childhood including physical, sexual or psychological abuse and psychological neglect (8), and stressful life events in adulthood such as separation from a partner or loss of a job (9). Also, both types of disorders show similar physiological dysregulations.

1.2 Somatic consequences of depressive and anxiety disorders

Besides often experienced somatic symptoms such as fatigue, increased hart rate, headaches, agitation or restlessness (7), depressive and anxiety disorders are increasingly recognized for their association with worse somatic health. Persons with depressive or anxiety disorders evidently show increased onset risks for several age-related somatic illnesses (10,11). Relative to the non-depressed population, persons with depression have an 80% increased onset risk of heart disease (12), 60% higher risk of diabetes (13), 58% of obesity (14), a 66% higher risk of Alzheimer's disease (15) and depression is even associated with a 29% increase in the chance of developing cancer (16). Also anxiety disorders are a risk factor for several somatic illnesses, with increased incidence rates of around 25% for coronary heart disease (17), diabetes (18) and disability (19). Further, having a depressive or anxiety disorder was found to be associated with excess mortality rates (20), with an estimated 10 years of potential life lost (21). Several physiological mechanisms have been proposed to explain this relationship: persons with depressive or anxiety disorder have an heightened immune response indicated by higher levels of pro-inflammatory cytokines (22-24); increased cortisol levels as a consequence of a dysregulated hypothalamus-pituitary-adrenal (HPA)-axis (25,26) and impaired autonomic nervous system functioning, including increased heart rate and decrease hart rate variability (27,28). A novel and intriguing suggestion is that persons with a depressive or anxiety disorder might be subject to accelerated biological aging processes, with alterations even on the cellular level. This may further explain their increased risks for developing age-related somatic conditions.

2. CELLULAR AGING

2.1 What is aging?

Aging can be described as the life-long accumulation of damage to the tissues, cells, and molecules of the body. Such damage, which is a consequence of the body's normal metabolism, consists of spontaneous errors in biochemical reactions, molecular damage by free radicals and progressive failure of cellular maintenance and repair systems; eventually leading to mutations, genomic instability, cell loss or altered intercellular communication (29). The body can tolerate this cellular and molecular damage to a certain extent; however, too much of it leads to age-related diseases, such as Alzheimer's or heart disease, and ultimately to death. Aging is generally considered a natural and evolutionary process. Dramatic increases in life expectancy – a 30-year increase in the 20th century, partly due to progress in public health and biomedical sciences – however, have led to wild claims about longevity or anti-aging strategies (30). Some go as far as stating that aging is a medical condition that has the potential to be treated, prevented or even reversed. Aubrey the Grey, co-founder of the SENS (Strategies for Engineered Negligible Senescence) Research Foundation, for example, states that we soon will be able to repair all types of molecular and cellular damage and that the first person who will live to 1,000 years has already been born (31,32). Others, instead, argue that the outlook of immortality is unlikely and has no place in a scientific discourse (30). Generally, the focus of scientific aging research is on increasing healthy lifespan and preventing pathological age-related conditions, rather than aiming for an increased maximum life span.

2.2 Markers of cellular aging

Studies of cellular aging may focus on various biomarkers (reviewed by López-Otín et al. (29)). In epidemiological research, the most widely used marker to study cellular aging is telomere length. Telomeres, from the Greek words *telos* (end) and *meros* (part), are non-coding DNA structures located at the ends of chromosomes. In 1978, Elizabeth Blackburn discovered that telomeres consisted of repetitive TTAGGG DNA sequences that provide chromosomal stability (33) (Figure 1). Further research showed that during every cell division, the final end of the telomere fails to be replicated, causing telomeres to become progressively shorter with age. This is due to the so-called "end-replication-problem", where DNA polymerase is unable to fully replicate the linear chromosome (34), which helps explain why cells have a limited capacity for replication (known as the Hayflick limit (35)). Critically short telomeres instigate the loss of telomere protective functions and eventually lead to cell cycle arrest and apoptosis pathways. Blackburn and Greider (36) later discovered the telomere extending enzyme telomerase (Figure 1), which is capable

of synthesizing telomeric DNA thereby compensating for the progressive telomere attrition. Most normal somatic cells, including most brain cells, have little telomerase, which reduces the cell's ability to maintain telomere length. Telomere length thus becomes progressively shorter with age (37) and therefore represents a biomarker of cellular age. Further, shortened telomeres have frequently been associated with various age-related somatic conditions such as cardiovascular disease (38) and diabetes (39) and with earlier mortality (40); although the degree to which they are causally involved in these conditions remains unknown.

Recent studies showed that approximately 64-70% of telomere length (42,43) and 28% of telomere attrition rate (43) is explained by genetic factors. However, telomere attrition is also thought to be influenced by environmental effects such as lifestyle, stress exposure, and physiological stress systems such as immune system activity or oxidative stress. Numerous *in vitro* and *in vivo* studies indeed found associations between short telomere length and higher levels of interleukin-6, C-reactive protein (44-46) and tumor necrosis factor-α (47), oxidative stress (48,49), hypocortisolism (50) or elevated catecholamines and cortisol (51,52), and metabolic abnormalities such as abdominal adiposity (53), adipose hypertrophy (54) and obesity (55,56). Furthermore, accelerated telomere shortening was related to higher levels pro-inflammatory cytokines (57), or telomere lengthening to lower levels of interleukin-6 (58). Overall, telomere length is thought to be a reflection of genetics, lifestyle factors and prior cumulative physiological stress exposure, and therefore serves as an indicator of biological age rather than chronological age.

Mitochondrial function is proposed to be another marker of cellular aging. Mitochondria are cellular energy-generating organelles that play an important role in adenosine triphosphate (ATP) production and regulation of apoptosis (59). Mitochondria have their own DNA, with genes that encode for essential components for ATP synthesis by oxidative phosphorylation (60). Each cell contains up to several thousand mitochondria in their cytoplasm, each containing multiple copies of mitochondrial DNA (61). A sufficient number of mitochondrial DNA molecules per cell – or mitochondrial DNA copy number - is found to be essential for healthy cellular function. Damage to mitochondrial DNA, as a results of reactive oxygen species (ROS) which cause oxidative damage, is postulated as one of the major causes of cellular aging (63). Accumulated damage to mitochondrial DNA may ultimately lead to age-related diseases (64) and a lower number of copies is cross-sectionally associated with chronic somatic diseases, such as hyperlipidaemia (65), Parkinson's disease (66), metabolic syndrome (67), and longitudinally with a higher risk of cognitive and physical decline and all-cause mortality (68).

Figure 1. Telomeres and telomerase

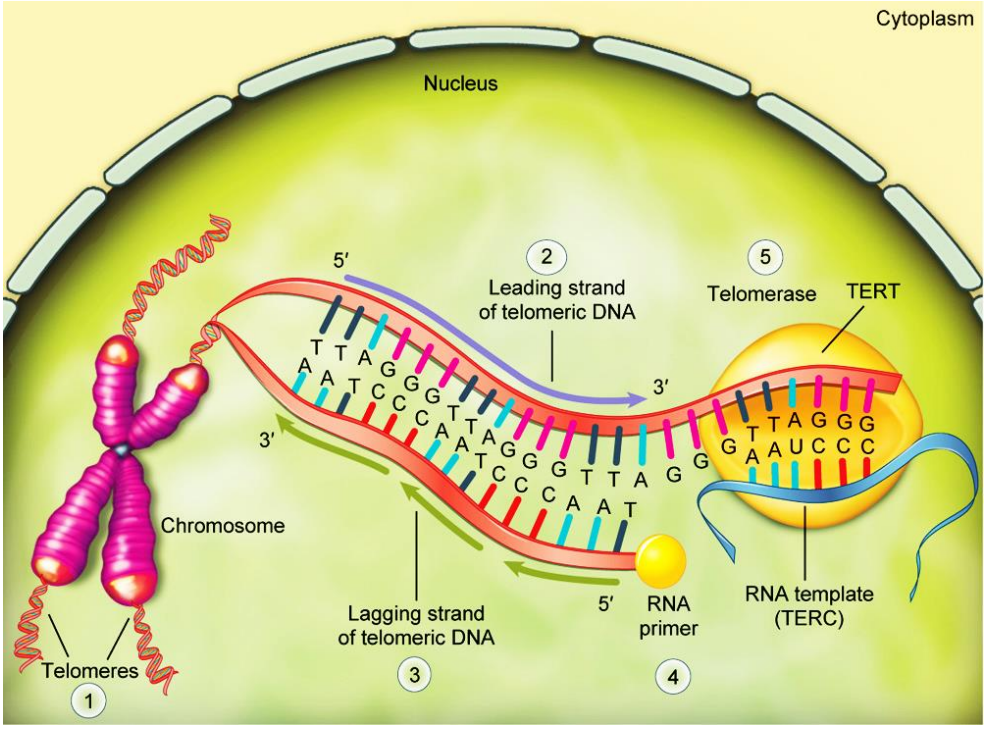

Figure legend. Telomeric TTAGGG DNA sequences [1] cap the ends of chromosomes and protect them from damage. Telomeres shorten with every cell division due to the so-called "end-replication problem" because DNA polymerase can only synthesize DNA in one direction (5' -> 3'). On the 5' -> 3' leading strand [2], this route is continuous, but on the lagging strand [3], it is discontinuous, synthesized in fragments that require a RNA primer molecule [4] to provide a 5' initiation point. As each fragment on the lagging strand (called "Okazaki fragments") is completed, the RNA primer translocates to initiate the synthesis of additional fragments. Since the RNA primer must always attach prior to the synthesis of the lagging strand fragments, and since the RNA primer must base pair to complementary nucleotides on the leading strand**,** the 5' end of lagging strand will always be shorter than the 3' end of the leading strand, and is thus incompletely replicated. The cellular enzyme telomerase [5] extends the telomeric DNA sequence at the ends of chromosomes. Telomerase is comprised of the telomerase reverse transcriptase (TERT) enzyme and a telomerase RNA component (TERC) that serves as a template for new complementary telomeric DNA construction along the leading strand. Figure and legend adapted from Lindqvist et al. (41)

3. ARE DEPRESSIVE AND ANXIETY DISORDERS ASSOCIATED WITH CELLULAR AGING?

The evident associations between depressive and anxiety disorders and age-related somatic conditions led to the hypothesis that depressed or anxious persons might actually age faster than their healthy counterparts. Innovative work from a collaboration of the departments of Biochemistry and Health Psychology of the University of California, San Francisco (UCSF) for the first time showed that chronic psychological stress was indeed related to markers of cellular aging, in particular shorter telomere length (69). Thereafter, Simon and colleagues (70) were the first to report on a relationship between shorter telomeres and affective disorders including depression. When comparing 44 non-depressed individuals with 44 mood disorder patients (MDD and bipolar disorder), they found that the latter group had shorter telomeres. This finding was replicated by Lung et al. (71) who found that 253 MDD patients had shorter telomere length than 411 community controls; by Hartmann et al. (72) showing shorter telomere length in 54 MDD patients compared to 20 healthy controls; and by Hoen et al. (73) who also found shorter telomeres for 206 MDD patients compared to 746 non-depressed persons in a sample of stable coronary heart disease patients. Wolkowitz et al. (49), however, found no overall difference in telomere length between 18 MDD subjects and 17 controls, while they did find a negative correlation between telomeres length and lifetime depression exposure. Only one study by Kananen et al. (74) examined the association between anxiety disorders and telomeres. They only found shorter telomeres in the older half of the anxiety disorder patients (>48 years old) and not in the whole sample of 321 patients compared with 653 controls. At the start of this thesis (January 2012) only few studies had thus been conducted on the association between telomere length and depressive or anxiety disorders, providing preliminary but inconsistent evidence for an association. Further, no studies on mitochondrial DNA in relation to depressive or anxiety disorders had been conducted.

4. COHORTS STUDIED IN THIS THESIS

4.1 The Netherlands Study of Depression and Anxiety (NESDA)

NESDA is a large ongoing study aiming to examine the course and consequences of depressive and anxiety disorders. A total of 2,981 participants were included at the baseline assessment which was performed between 2004-2007. This sample consists of persons between 18 and 65 with a current depressive or anxiety disorder (57%), persons with a remitted disorder (21%) and controls with no lifetime history of any psychiatric disorder (22%). Participants were recruited from the community, primary care and through specialized mental health care, in order to include a representative sample of

depressed and anxious persons. Exclusion criteria were insufficient command of the Dutch language, and a primary clinical diagnosis of bipolar disorder, obsessive-compulsive disorder, posttraumatic stress disorder (PTSD), severe substance use disorder or a psychotic disorder. Participants were assessed during a 4-hour clinic visit. Every two years after the baseline assessment, face-to-face follow-up assessments were conducted. Follow-up assessments had a response of 87.1% (N=2596) at two-year follow-up, 80.6% (N=2402) at four-year follow-up and 75.7% (N=2256) at six-year follow-up. More details of NESDA's methods and study sample have been described in the design paper by Penninx et al. (75).

4.2 The Netherlands Study of Depression in Older Persons (NESDO)

The NESDO study is a prospective study that aims to investigate the natural course, determinants, and consequences of late-life depression. From 2007 until 2010, NESDO included 510 persons aged 60 to 93 years, divided into 378 persons with a DSM-IV based depressive disorder (current MDD, dysthymia or minor depression) and 132 never depressed comparisons. Participants were recruited through outpatient and inpatient mental healthcare institutions and general practices. Exclusion criteria were being unable to provide written informed consent; not fluent in Dutch; a primary diagnosis of dementia, a Mini-Mental State Exam score below 18, or clinician-suspected dementia. A detailed description of the NESDO study is provided by Comijs et al. (76).

4.3 The Coronary Artery Risk Development in Young Adults Study (CARDIA)

CARDIA is a study examining the development and determinants of clinical and subclinical cardiovascular diseases and their risk factors. During 1985 to 1986, CARDIA performed community-based recruitment of 5,115 research study participants in Birmingham, AL, Chicago, IL, and Minnespolis, MN and from the membership of a prepaid health care plan in Oakland, CA. The study sample was balanced by race, sex and education. Follow-up examinations were conducted at years 2, 5, 7, 10, 15, 20, and 25. A majority of the group has been examined at each of the follow-up examinations (90%, 86%, 81%, 79%, 74%, 72%, and 72%, respectively). CARDIA administered the self-reported Center for Epidemiologic Studies Depression (CES-D) scale as a measure depressive symptoms (77). Other details of study design, recruitment, and procedures have been published elsewhere (78).

5. AIMS AND OUTLINE OF THIS THESIS

The overall objective of this thesis is to study associations between markers of cellular aging and depressive and anxiety disorders. In particular, the first aim was to discover whether telomere length is cross-sectionally associated with MDD and anxiety disorders. In **Chapter 2** we describe the cross-sectional associations of telomere length with MDD diagnosis status and further depression characteristics in NESDA. **Chapter 3** describes similar cross-sectional associations in the NESDA sample of telomere length with anxiety disorders and characteristics. In **Chapter 4** the association between telomere length and MDD is described in older adults from the NESDO study. **Chapter 5** comprises a meta-analysis that examines the cross-sectional relationships between telomere length and depression, anxiety disorders, PTSD, bipolar disorder and psychotic disorders.

The second aim of this thesis was to determine whether telomere length is associated with established risk factors of depressive and anxiety disorders: childhood trauma and recent stressful life events. Results from the NESDA study relating to this second aim are described in **Chapter 6**.

The third aim was to provide insight in the longitudinal trajectories of depressive and anxiety disorders and telomere length and mitochondrial DNA copy number. First, **Chapter 7** consists of a review paper that discusses the hypothesis of reversibility of cellular aging in depression. **Chapter 8**, further, examines the six-year longitudinal relationship of telomere length with depressive and anxiety disorders in NESDA. Subsequently, in **Chapter 9** the longitudinal associations of depressive symptoms with telomere length and mitochondrial DNA over ten years are described using the CARDIA sample.

Our fourth and final aim was to shed more light on possible underlying mechanisms of the association between telomere length and depressive and anxiety disorders. In **Chapter 10** we examine the extent to which physiological stress systems are related to telomere length and in **Chapter 11** we test the extent to which physiological stress systems, metabolic dysregulations and lifestyle factors mediate the relationship between depressive and anxiety disorders and telomere length. Finally, **Chapter 12** summarizes the main findings of this thesis and discusses their implications.

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